Volume 14 Article 40

4-1-1987

Research Notes : United States : Necrotic root mutants in a genetically unstable line of soybean

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1) New mutations in a genetically unstable line of soybean.

Most plants of the Asgrow Mutable line of soybean are chimeric for flower color (Groose and Palmer, 1986). Mutable plants produce both entirely near-white and entirely purple flowers, as well as flowers of mutable phenotype with purple sectors on near-white petals. This line carries an unstable recessive ('mutable') allele of the *w4* locus that conditions anthocyanin pigmentation (Weigelt et al., 1986). The mutable allele reverts at high frequency from the recessive state to a stable dominant state. Many such mutable alleles in plants have been analyzed at the molecular level and in every instance the action of a transposable element has been established (Döring and Starlinger, 1986). We hypothesize that the Asgrow Mutable line harbors an active transposable element system and that the high frequency of reversion of the unstable allele results from excision of the putative element from the *w4* locus.

The objective of our study was to recover new mutations at other loci in the Asgrow Mutable line as evidence for transposition of a mobile genetic element. We reasoned that the probability of recovering new mutations might be maximized by searching for mutants among progenies of wildtype (germinal revertant) progeny of mutable plants. A germinal revertant is the result of a reversion of the unstable allele in the germline of a mutable parent. Germinal revertants produce only wildtype purple flowers and their self progenies either breed true for wildtype pigmentation or segregate 3 wildtype:1 mutable. If a reversion of the unstable allele is the result of transposition of the positive element out of the *w4* locus and into another locus, then new mutations at other loci might be detected among the progenies of germinal revertants. Our strategy was to survey progenies of many germinal revertants (each of which was derived from an independent reversion event) for new mutations at as many loci as possible.

A summary of the study is presented in this communication. Three accompanying research notes describe new mutations for chlorophyll deficiency (Groose et al., 1987), partial sterility (Groose and Palmer, 1987) and necrotic roots (Blomgren et al., 1987).

Materials and methods: The experiment was conducted as follows:
Step 1: (F_9 generation; Field nursery, Ames, Iowa, 1985). Two thousand mutable plants were selected from F_9 progeny rows that descended from 60 highly mutable F_8 plants of the Asgrow Mutable line. Each F_9 plant was threshed separately to produce 2000 F_{10} families.

Step 2: (F_{10} generation; Off-season nursery, Puerto Rico, winter 1985-86). Approximately 30 seed of each of the 2000 F_{10} families were planted to produce an F_{10}-progeny row. A single germinal revertant was selected from each row that contained at least one germinal revertant (1599 rows). Selection of a single germinal revertant from each progeny row assured that every germinal revertant was derived from an independent reversion event. For each row that produced no germinal revertants (401 rows), a single mutable plant was selected. Selected plants were threshed separately to produce 2000 F_{11} families.

Step 3: (F_{11} generation; Greenhouse sandbench and field nursery, Ames, Iowa, 1986). Sufficient seed was available to test 1936 and 1697 F_{11} families, respectively, in a greenhouse sandbench and in a field nursery. In the sandbench, approximately 25 seedlings in each family were observed for segregation for new mutations until the second trifoliolate leaf stage when plants were pulled from the bench for examination of root systems. In the field nursery, approximately 25 plants in each family were observed periodically throughout the season and at maturity for segregation for new mutations. In both locations, progenies were surveyed for characters that are easily evaluated by visual examination. These included chlorophyll pigmentation, root fluorescence, seed pigmentation, leaf form, sterility, dwarfness, stem and petiole morphology, and time of flowering and maturity. Dominant alleles of more than 50 described nuclear loci condition the wildtype phenotypes of these traits (Palmer and Kilen, 1987) and the Asgrow Mutable line breeds true for wildtype for all these traits.

Most new mutations that result from insertion of a transposable element are expected to be recessive. In this experiment, some F_{10} plants are expected to descend from F_9-germline sectors that carry new recessive mutations. Mutant F_9-germline sectors are expected to be heterozygous for the new mutations and to produce F_{10} progeny that segregate 1 homozygous wildtype:2 heterozygous:1 homozygous recessive. Therefore, 25%, 50%, and 25%, respectively, of F_{11} families that descend from F_9-germline sectors with new nonlethal recessive mutations are expected to breed true for wildtype, segregate 3 wildtype:1 recessive, and breed true for the recessive phenotype. Deleterious mutations are expected to eliminate some homozygous recessive plants, alter segregation ratios, and reduce the probability of recovering F_{11} families that breed true for recessive mutations.

Results: Several new mutations were either true-breeding or segregating in the F_{11} (Table 1). Each of these was recovered in a different F_{11} family. All were derived from germinal revertant F_{10} plants and probably descend from mutational events in germline sectors of mutable F_9 plants. These mutations are described in more detail in the accompanying research notes.

In addition, possible new mutations were recovered as single variant plants in several other F_{11} families (Table 2). If any of these variant plants is the result of a genetic mutation, the mutational event probably occurred in a germline sector of the F_{10} parent. Inheritance of these possible new mutations is the subject of current research.

Discussion: We have identified an array of new mutations in the Asgrow Mutable line. Molecular genetic analysis of these materials is underway. If an inserted element is identified at the mutable allele of the *w4* locus, it should be possible to clone a molecular probe from the element and analyze the new mutations described in the present study. Cosegregation of DNA polymorphisms homologous to the probe with any of these new mutations would provide proof of transposition of the element in the genome.

Acknowledgment: We thank José Bravo for his assistance at the Asgrow Seed Co. winter soybean nursery at Isabela, Puerto Rico.

Table 1. Summary of new mutations recovered as true-breeding or segregating in F_{11} families of the Asgrow Mutable line

Mutation	Description
Chlorophyll deficient #1	Segregating for normal and variegated chlorophyll pigmentation. Variegated plants have normal and delayed-green sectors.
Chlorophyll deficient #2	Segregating (approximately 3 wildtype:1 mutant) for normal and delayed-green chlorophyll pigmentation.
Chlorophyll deficient #3	Same as Chlorophyll deficient #1.
Partial sterile #1	True-breeding for reduced number of seed per pod.
Partial sterile #2	Segregating for plants producing normal and reduced numbers of seed per pod.
Partial sterile #3	Same as Partial sterile #2.
Partial sterile #4	Same as Partial sterile #2.
Necrotic root #1	Segregating (approximately 3 wildtype:1 mutant) for plants with normal and necrotic root systems.
Necrotic root #2	Same as Necrotic root #1.
Necrotic root #3	Same as Necrotic root #1.

Table 2. Summary of possible new mutations recovered as single plants in F_{11} families of the Asgrow Mutable line

Mutation	Description
Chlorophyll deficient #4	Variegated with normal green and yellow sectors.
Chlorophyll deficient #5	Yellow.
Chlorophyll deficient #6	Green with one yellow unifoliolate.
Chlorophyll deficient #7	Variegated with normal green and delayed-green sectors.
Chlorophyll deficient #8	Variegated with normal green and yellow sectors in one unifoliolate.
Nearly sterile	Almost completely sterile except for several normal pods.

References

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2) Chlorophyll-deficient mutants in a genetically unstable line of soybean.

Mutations for chlorophyll deficiency were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element. A detailed protocol for the experiment is presented in the preceding research note (Groose and Palmer, 1987). Each of three independent mutations that are described below was discovered in a sandbench test of a different F_{11} family, each of which had descended from a different F_9 plant of the Asgrow Mutable line.

Chlorophyll deficient #1: In the sandbench test of this F_{11} family, 30 seedlings were normal green (wildtype) and four seedlings were variegated for normal and delayed-green sectors. In newly-opened leaves, chlorophyll-deficient sectors were yellow. As leaves fully expanded, however, deficient sectors became nearly normal green.

In the field nursery, chlorophyll-deficient sectors were not detected on any of 24 plants of this F_{11} family. These plants were threshed separately to produce 24 F_{12} families and approximately 30 seed of each F_{12} family were planted in the greenhouse sandbench. Seedlings were examined frequently for chlorophyll-deficient sectors until the second trifoliolate leaf stage. Overall, 497 (84.1%) seedlings were normal, 73 (12.4%) seedlings were variegated for normal and delayed-green sectors, and 21 (3.6%) seedlings were entirely delayed-green. Although chlorophyll deficiency had not been detected

in any of the 24 field-grown F_{11} parents, chlorophyll-deficient seedlings (either variegated or entirely delayed-green) were found in 19 of the 24 F_{12} progenies. Five F_{12} families produced only normal green seedlings. A chi-squaretest for homogeneity of the distribution of normal and chlorophyll-deficient individuals among F_{12} families was performed. Variegated and entirely delayed-green seedlings were considered together as a single class because entirely delayed-green seedlings were relatively rare. The test for homogeneity indicated differential production of normal and chlorophyll-deficient progeny by the F_{11} parents ($\chi^2 = 123.665$, 23 df, $P < 0.005$). The mode of inheritance of this unstable trait remains to be determined.

Chlorophyll deficient #2: In the sandbench test of this F_{11} family, 23 seedlings were normal green (wildtype) and nine seedlings were delayed-green. Newly-opened leaves of delayed-green seedlings were yellow but became nearly normal green as leaves fully expanded. No variegated seedlings were observed and it appeared that this family was segregating for a stable recessive mutation. This mutation appears to be deleterious. Although mutant seedlings eventually attained nearly normal chlorophyll pigmentation, they were stunted relative to normal segregates.

In the field nursery, chlorophyll-deficient plants had not been noticed among 31 plants of this F_{11} fmaily. Subsequent to the discovery of the mutation in the sandbench test, a close examination of the field-grown plants indicated that 13 of the 31 plants were slightly paler green and were stunted relative to normal plants of the Asgrow Mutable line. These were classified as "possible mutants" (recessive homozygotes). One of these died before maturity. The thirty plants that survived to maturity were threshed separately to produce 30 F_{12} families.

The F_{12} families were used to test the hypothesis that the F_{11} family was descended from an F_{10} parent that was heterozygous for a new single gene recessive mutation. Approximately 30 seed of most F_{12} families were planted in the greenhouse sandbench. Field-grown plants that had been classified as possible mutants produced less than 30 seed per plant. The sandbench test revealed that five, twenty, and five F_{12} families, respectively, were true-breeding wildtype, segregating, and true-breeding delayed-green. This segregation fit the 1:2:1 ratio expected for F_{11} progeny of an F_{10} parent heterozygous for a single gene mutation ($\chi^2 = 3.333$, 2 df, $0.100 < P < 0.250$).

Analysis of segregation within the 20 segregating F_{12} families provided additional evidence that the "chlorophyll deficient #2" is conditioned by a recessive mutation at a single locus. Seedlings in these families segregated at the 3:1 ratio expected for F_{12} progeny of heterozygous F_{11} parents (Table 1).

Table 1. Segregation of seedlings (normal:chlorophyll deficient) within F_{11}-derived F_{12} families segregating for "Chlorophyll deficient #2"

	Normal	Chlorophyll deficient	χ^2 (3:1)	P
Totals	357	105	16.882	
Pooled chi-square (1df)			1.273	.25-.50
Homogeneity chi-square (19 df)			15.549	.50-.75

All five F_{12} families that were true breeding for delayed-green were descended from F_{11} plants that had been classified as "possible mutants." The other surviving F_{11} "possible mutants" proved not to be recessive homozygotes. Thus, field evaluation of this trait proved to be somewhat unreliable. Recessive homozygotes were always pale green and stunted but the converse was not always true.

Chlorophyll deficient #3: In the sandbench test of this F_{11} family, 16 seedlings were normal green (wildtype) and eight seedlings were variegated for normal and delayed-green sectors. Variegated seedlings were identical to variegated seedlings of "Chlorophyll deficient #1" except that delayed-green sectors tended, on average, to be larger than delayed-green sectors produced on variegated plants of "Chlorophyll deficient #1." For example, entirely delayed-green unifoliolate leaves were produced on several variegated plants.

In the field nursery, chlorophyll-deficient sectors were not detected on any of 16 plants of this F_{11} family. These plants were threshed separately to produce 16 F_{12} families which were studied in the same way as the F_{12} families of "Chlorophyll deficient #1." Overall, 359 (90.9%) seedlings were normal, 26 (6.6%) seedlings were variegated for normal and delayed-green sectors, and 10 (2.5%) seedlings were entirely delayed-green. Although chlorophyll deficiency had not been detected in any of the 16 field-grown F_{11} parents, chlorophyll-deficient seedlings were found in 9 of the 16 F_{12} progenies. Seven F_{12} families produced only normal green seedlings. The chi-square test for homogeneity of the distribution of normal and deficient individuals among F_{12} families indicated differential production of normal and deficient progeny by the F_{11} parents ($\chi^2 = 72.300$, 15 df, $P < 0.005$). Like "Chlorophyll deficient #1," the mode of inheritance of this unstable trait remains to be determined. It is interesting that these three mutations for chlorophyll deficiency are all characterized by a delayed-green phenotype. We are currently entertaining the hypothesis that these are three independent mutations (one stable and two unstable) of the same nuclear gene.

Reference

Groose, R. W. and R. G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genet. Newsl. 14: 164-167.

R. W. Groose - USDA
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3) Partially sterile mutants in a genetically unstable line of soybean.

Mutations for partial sterility were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element (Groose and Palmer, 1987). The mutations were discovered in a field nursery that included 1697 F_{11} progeny rows of the Asgrow Mutable line. Mutant plants with reduced numbers of seeds per pod were first detected when the nursery was examined at maturity. Each of the four independent mutations for partial sterility was discovered in a different F_{11} family, each of which had descended from a different F_9 plant of the Asgrow Mutable line.

Normal plants of the Asgrow Mutable line produce mostly three- and two-seeded pods. A random sample of plants taken from throughout the nursery produced one-, two-, three-, and four-seeded pods as indicated in Table 1. Plants of a distinctly different type were found in four F_{11} families. These plants were characterized by a reduced number of seeds per pod and were designated "partial steriles." Partial sterile plants produce a preponderance of one- and two-seeded pods (Table 1) and are easily recognized at maturity by the large number of one-seeded pods. Partial sterile plants were otherwise identical to normal plants. The reduction in number of seeds per pod appeared to be the result of ovule or very early embryo abortion.

Table 1. Number and percentage of four different types of pods produced by normal and partial sterile mutant F_{11} plants of the Asgrow Mutable line

		Type of pod							
		One-seeded		Two-seeded		Three-seeded		Four-seeded	
Entry	No. plants	No.	%	No.	%	No.	%	No.	%
Asgrow Mutable line[a]	16	118	10.6	466	41.8	521	46.7	10	0.9
Partial sterile #1[b]	12	514	44.1	511	43.9	139	11.9	1	0.1
Partial sterile #2[c]									
Normal plants	9	84	12.6	276	41.5	296	44.5	9	1.4
Partial steriles	11	423	47.1	388	43.2	87	9.7	0	0.0
Partial sterile #3[c]									
Normal plants	3	35	14.5	99	41.1	105	43.6	2	0.8
Partial steriles	12	507	50.3	418	41.5	82	8.1	1	0.1
Partial sterile #4[c]									
Normal plants	8	50	10.4	176	36.5	244	50.6	12	2.5
Partial steriles	12	335	48.6	293	42.5	60	8.7	1	0.1

[a]Random sample of F_{11} plants from throughout the nursery.

[b]All plants in this F_{11} family were partial sterile.

[c]This F_{11} family was segregating for normal and partial sterile plants.

All plants in the F_{11} family in which "Partial sterile #1" was discovered were partial steriles. The families in which "Partial sterile #2," "Partial sterile #3," and "Partial sterile #4" were discovered were segregating for normal and partial sterile plants. Normal plants in these families produced one-, two-, three-, and four-seeded pods in essentially the same proportions as the random sample of plants taken from throughout the nursery. Partial sterile plants in these families produced one-, two-, three-, and four-seeded pods in essentially the same proportions as plants of "Partial sterile #1." The mechanism and inheritance of partial sterility in these materials is the subject of current research.

Reference

Groose, R. W. and R. G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genet. Newsl. 14: 164-167.

R. W. Groose - USDA
R. G. Palmer - USDA

4) Necrotic root mutants in a genetically unstable line of soybean.

Mutations for necrotic roots were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element (Groose and Palmer, 1987). Each of three independent mutations for necrotic roots was discovered in a different F_{11} family, each of which had descended from a different F_9 plant of the Asgrow Mutable line.

Seedlings of 1936 F_{11} families were pulled from the sandbench at the second trifoliolate leaf stage and roots were examined for nonfluorescent root mutations. Seedlings of most soybean lines, including the Asgrow Mutable line, fluoresce under UV light. Root fluorescence is conditioned by several loci (Sawada and Palmer, 1987). Putative nonfluorescent mutants were identified in three F_{11} families. However, close examination of the mutants revealed that the failure of the roots to fluoresce was not the result of mutation at any of the loci for root fluorescence. The apparent lack of fluorescence in these mutants was due to an accumulation of a reddish-brown slime that obscured an underlying fluorescent root surface. The root systems of these mutants were weak and necrotic. Several mutants were transplanted to pots in the greenhouse but eventually most died. Survivors produced very few progeny. It is unlikely that plants with this trait would survive to maturity in the field.

In the sandbench test of the three F_{11} families, seedlings segregated approximately 3 wildtype:1 necrotic (Table 1). We hypothesized that each of the F_{11} families was segregating for a new recessive mutation for necrotic roots and tested the hypothesis with F_{11}-derived F_{12} families. For each of the three F_{11} families, plants that had been grown in a field nursery were threshed separately and their F_{12} progenies were tested in the sandbench. In each case, approximately one-third of F_{12} families were true-breeding for normal roots and approximately two-thirds of F_{12} families were segregating for normal and necrotic root (Table 2). In each case, the observed segregation fit the 1:2 ratio expected for F_{11} progeny of an F_{10} parent heterozygous for a lethal recessive mutation.

Analysis of segregation ratios within F_{12} families provided additional insight into the behavior of the necrotic root trait. F_{12} families derived from heterozygous F_{11} parents are expected to segregate 3 normal:1 necrotic in the sandbench test. For all three mutations, a small excess of seedlings was observed in the necrotic root class (Table 3). In the case of "Necrotic root #3", the deviation from the expected 3:1 was significant at the P=0.01 level. At present, we believe that the excess in the recessive class is easily explained as the result of misclassification. In the sandbench, a proportion of seedlings of any soybean line will die from a variety of causes.

Roots of such seedlings may mimic the necrotic root trait. In this experiment it is likely that some seedlings that were not homozygous recessive for the necrotic root trait were misclassified as "necrotic root," In other words, classification for this trait is not expected to be perfectly reliable and a slight deviation from the 3:1 might be expected. On the whole, we believe that the results of this experiment support the conclusion that "Necrotic root #1," "Necrotic root #2," and "Necrotic root #3" each resulted from a recessive mutation at a single locus. Genetic analysis is in progress to determine if these three independent mutations are allelic to the same locus.

Table 1. Segregation of seedlings (normal root:necrotic root) in three F_{11} families of the Asgrow Mutable line

	Necrotic root #1		Necrotic root #2		Necrotic root #3	
	Normal	Necrotic	Normal	Necrotic	Normal	Necrotic
F_{11} seedlings	37	12	21	7	33	12
Chi-square (3:1, 1df)	0.007		0.000		0.067	
Probability	0.90-0.95		0.995-1.000		0.75-0.90	

Table 2. Ratio of F_{11}-derived F_{12} families (true-breeding normal:segregating) for three necrotic root mutations recovered in the Asgrow Mutable line)

	Necrotic root #1		Necrotic root #2		Necrotic root #3	
	True breeding normal	Segregating	True breeding normal	Segregating	True breeding normal	Segregating
F_{12} families	4	15	8	10	6	11
Chi-square (1:2, 1df)	1.286		1.000		0.029	
Probability	0.25-0.50		0.25-0.50		0.75-0.90	

Table 3. Segregation of seedlings (normal root:necrotic root) within segregating F_{11}-derived F_{12} families for three necrotic root mutations recovered in the Asgrow Mutable line

	Necrotic root #1		Necrotic root #2		Necrotic root #3	
	Normal	Necrotic	Normal	Necrotic	Normal	Necrotic
Total F_{12} seedlings	260	103	197	78	214	98
% F_{12} seedlings	71.6	28.4	71.6	28.4	68.8	31.4
Pooled chi-square (3:1, 1df)	2.227		1.660		6.837	
Probability	0.10-0.25		0.10-0.25		0.005-0.010	
Homogeneity chi-square	13.356 (14df)		17.871 (9df)		11.183 (10df)	
Probability	0.25-0.50		0.025-0.050		0.25-0.50	

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5) Monosomics from synaptic KS mutant.

Our study of unknown synaptic mutants attempts to provide more information about genetics of megasporogenesis and microsporogenesis in soybean. This study attempts also to develop aneuploid stocks to be used to establish a genetic map in soybeans. Synaptic mutants are known to produce aneuploid and polyploid plants. We believe that variability in genetic background of new synaptic mutants can be helpful in obtaining all 20 possible primary trisomics and other aneuploids in soybeans.

Materials and methods: Sterile plants were found in the F_3 progeny from a cross between two dwarf plants (T210 *df2* x dwarf mutant 35-1-2) from material obtained from K. Sadanaga. F_3 progeny segregated 41 fertile to 15 sterile plants. Forty F_4 KS progenies were observed for sterility vs. fertility segregation in 1985. For aneuploid identification, the method described by Palmer and Heer (1973) was used for chromosome number determination. I_2KI stain was used for estimation of pollen fertility/sterility.

Results and discussion: Among 40 F_4 progenies, 13 did not segregate; 27 progenies segregated for sterility. Among segregated progenies, 915 plants were fertile and 330 plants were sterile (Table 1). These results indicated that a single locus was conditioning female and male sterility.

Table 1. Segregation for fertility vs. sterility in F_4 population of KS synaptic mutants

Observed number of plants			Expected number of plants	
Fertile	Sterile	Total	Fertile	Sterile
915	330	1245	933.75	311.25
χ^2 3:1	1.53			
P	0.25 - 0.10			

KS mutation was highly female and male sterile. Only ten seeds were harvested from all sterile plants (0.03 seed per plant). Nine seeds germinated and seedlings were checked for chromosome number. In the progeny of sterile plants, diploids, addition aneuploids, deficiency polyploids and, what was the most valuable, a deficiency aneuploid 39-chromosome plant, No. KS-6, were identified (Table 2).

Original monosomic - KS-6: The monosomic was grown during winter/spring seasons in the USDA greenhouse. We noticed partial sterility of this plant, 24.7% nonviable pollen grains (Table 3). As far as the female reproduction is concerned, the number of abortions was higher than number of harvested seeds. Especially high frequency of ovule abortion was noted (Table 3). This original monosomic produced 130 seeds.

Table 2. Chromosome number of progeny of the KS sterile mutant

Entry No.	No. of seeds	No. of chromosomes	Fertility
S85-22	1	44	Fertile
S85-26	1	42	Fertile
S85-28	1	44	Died
S85-29	1	40	Fertile
S85-32	1	39	Partially sterile
S85-33	2	45	Fertile
		72	Sterile
S85-34	1	41	Sterile
S85-37	1	43	Sterile

Table 3. Fertility of the original 39-chromosome plant KS-6

Traits	Number	Percentage
Stained pollen grains	870	75.3
Nonstained pollen grains	285	24.7
Number of seeds	130	41.1
Number of abortions	186	58.9
Aborted embryos	6	--
Aborted ovules	180	--
Ratio seeds:abortions	0.7	--
Ratio of aborted embryos:ovules	0.03	--

Progeny of original monosomic KS-6: Of the 1380 seeds, 94 germinated; their chromosome numbers were determined. Ninety-two seedlings were diploids, two seedlings, KS-6-21 and KS-6-26 (Fig. 1), had 39 chromosomes. Transmissions of n-1 gametes was very low; frequency of monosomics was 2.1%. We did not find any 2n + 1 genotypes. KS-6-21 and KS-6-26 plants were grown during summer 1986 in the greenhouse. Plant KS-6-21 had only 3% sterile pollen grains; plant KS-6-26 had 17.1% sterile pollen grains (Table 4). From a comparison of pollen fertility and seed set in several species, abnormalities of female reproduction were considered to be less than on the PMC side. However, in some species (*Oriza sativa, Brassica oleracea* var. *capitata*), a stronger effect of factors causing disturbances in cell division was observed on megasporogenesis than on microsporogenesis (Katayana, 1964; Gottschalk and Konvicka, 1971). In observed monosomics, the percentage of abnormalities on the female side was higher than on the male side. Low transmission of n-1 gametes from the original monosomic, high rate of abortions of KS-6-21 and of KS-6-26 make these plants unreliable as sources of monosomics by sexual reproduction.

Soybeans can tolerate addition aneuploids and set seeds. Beversdorf and Bingham (1975) found addition aneuploids among the normally-shaped seeds of 40-chromosome plants derived from 43-44-chromosome plants. A few trisomics

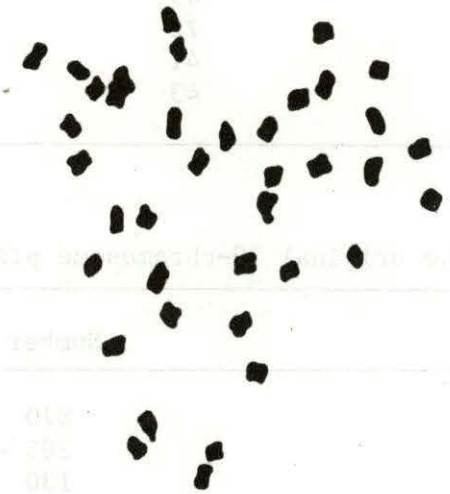

Figure 1. Metaphase of 39-chromosome soybean, KS 6-26.

Table 4. Fertility of 39-chromosome plants derived from KS-6

Traits	KS-6-21	KS-6-26
Stained pollen grains	490	1062
Nonstained pollen grains	15	219
Percentage of sterile pollen grains	3.0	17.1
Number of seeds	108	121
Number of abortions	91	105
Percentage of abortions	45.7	46.5
Number of aborted embryos	35	42
Number of aborted ovules	56	63
Ratio seeds:abortions	1.2	1.1
Ratio seeds:ovules	0.6	0.7

were obtained by using haploids, but no monosomics or other deficiency aneuploids have been confirmed (Sorrels and Bingham, 1978). In chromosome transmission of n + 1 gametes, soybeans behave similarly to known polyploids. Ovule transmission averages 34% for Tri A, 45% for Tri B, 39% for Tri C, and pollen transmission rates were respectively 27%, 22%, and 43% (Palmer, 1972). It is difficult to find an explanation why viable n-1 gametes are so rarely produced.

A system found in corn produces a high frequency of monosomics. They are generated by r-X1 deficiency, a submicroscopic deficiency including the R locus on chromosome 10 (Weber 1970, 1973, 1982). To establish a genetic map of soybeans, we have to search for an efficient system of obtaining monosomics, as well as to search for new translocations.

In our study, monosomics were fertile or partially sterile. Progeny of the original monosomic segregated for fertility vs. sterility and gave a good fit to 3:1 ratio (Table 5). These observations showed that the KS sterile mutation is not located on the missing chromosomes.

Table 5. Segregation for fertility vs. sterility of the progeny of original monosomic KS-6

Observed number of plants			Expected number of plants	
Fertile	Sterile	Total	Fertile	Sterile
68	24	92	69	23
χ^2 (3:1)	0.057			
P	0.90 - 0.75			

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6) New male-sterile, female-fertile mutations in soybeans.

Three new spontaneous independent male-sterile, female-fertile (MS-FF) mutations were used for cytological observations and for allelism tests. One of them was found in the cultivar 'Beeson'. The JB and BDI mutations were found in advanced breeding populations. The Beeson, JB, and BDI mutants produced sterile plants, which at the time of anthesis have shrunken, distorted anthers. These sterile plants can be differentiated easily from fertile plants by examining the flowers with magnifying glasses. Degenerated microspores were not released from anthers. Male sterile-female fertile mutant *ms2 ms2* has similar anther features.

Methods: Beeson, JB, and BDI mutants were crossed with *ms2* genotypes. We made crosses among the new mutants as well. In most crosses, sterile plants were used as female parents and known heterozygotes were used as male parents. In cross number 1 (Table 1) and number 5 (Table 2), genotypes of male parent were unknown. They could be dominant homozygotes or heterozygotes of sterility genes.

Table 1. Fertility of F_1 plants from crosses of unknown male-sterile, female-fertile mutants with the *ms2* genotype

Cross	Number of plants	
	Fertile	Sterile
1. *ms ms* BDI x *Ms2 Ms2*, *Ms2 ms2*	46	0
2. *ms2 ms2* x *Ms ms* BDI	20	0
3. *ms ms* Beeson x *Ms2 ms2*	46	0
4. *ms ms* JB x *Ms2 ms2*	28	0
5. *ms2 ms2* x *Ms ms* JB	17	0

Table 2. Fertility of F_1 plants among unknown male-sterile, female-fertile mutants

Cross	Number of plants	
	Fertile	Sterile
1. *ms ms* Beeson x *Ms ms* BDI	49	0
2. *ms ms* JB x *Ms ms* BDI	45	0
3. *ms ms* BDI x *Ms ms* JB	10	0
4. *ms ms* Beeson x *Ms ms* JB	13	0
5. *ms ms* Beeson x *Ms Ms* JB, *Ms ms* JB	16	0
6. *ms ms* JB x *Ms ms* Beeson	37	0

F_1 seeds were planted in the Iowa State University-University of Puerto Rico Soybean Breeding Nursery, Isabela, Puerto Rico. During flowering, buds were collected for pollen viability evaluation.

Results and discussion: We obtained sufficient number of seeds from test crosses to get information about allelism of the new mutations.

F_1 plants from crosses of BDI, Beeson, and JB mutations with *ms2* genotype were fertile, indicating that none of these new mutations are located at the *ms2* locus.

F_1 plants from crosses made among new mutations also were fertile, indicating that they are non-allelic to each other.

It is interesting that in soybeans one locus (*ms1* locus) has six independent mutations (Skorupska and Palmer, 1986), the ms_3 was found to have two mutations (Graybosch and Palmer, 1987), and, conversely, different alleles can produce the same phenotypic effect but are located at different loci.

Our observations have shown that the JB mutation is closely linked (2.48-3.25% of recombination) to the *w1* locus (Skorupska and Palmer, 1986). We crossed the BDI, Beeson and JB mutants with the satellite trisomic. The purpose of this aspect of our study was to find if new MS-FF mutations are located in linkage group 8. This material is now under investigation. We can expect positive results as far as the JB mutant is concerned, because the *w1* gene is on the satellite chromosome (Sadanaga and Grindeland, 1984). If data show that BDI and Beeson mutations are not in LG8, we will be able to avoid testing of locus-to-locus linkage with genes located in LG8.

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7) New independent mutation: *ms1* (Ames 2).

Five different populations have been recognized as a source of *ms1* alleles. Genetics studies of male-sterile, female-fertile mutations conducted by Palmer et al. (1978) showed that *ms1* (North Carolina) (T260), *ms1* (Urbana) (T266), *ms1* (Tonica) (T267), and *ms1* (Ames) (T268) are independent mutations at the *ms1* locus. Yee and Jian (1983) reported another mutation at the *ms1* locus, designated Shennong Male-Sterile Soybean L-78-387.

Sterile plants setting very few pods were found by Ron Secrist in his plant breeding nursery in 1984. Our previous observations based upon F_1 allelism tests indicated that this unknown mutant was allelic to *ms1* (T266) (Skorupska and Palmer, 1986). This report reviews the origin of the mutant and gives F_1 and F_2 results.

Origin of unknown mutant. Plant breeding material was derived from 1979 to 1984 from 100 seeds of the Soybean Germplasm Population, AP6 (S1) C1 (see Figure 1, Skorupska and Palmer, 1986). According to Ron Secrist, *ms1 ms1* plants have never been grown in his nursery and the populations had no chance for contamination with the *ms1* allele.

Methods. Crosses were made in 1985 using homozygous recessive *ms1 ms1* (T266) plants as female parents and heterozygotes of the new mutant as male parents. F_1 seeds were planted in the ISU Soybean Breeding Nursery in Isabela, Puerto Rico, and in the USDA greenhouse in Ames. Thirty-eight F_1 plants were obtained for allelism tests. They were classified for male sterility/fertility on the basis of pollen staining in I_2KI (Table 1). F_1 progenies were grown in Ames in 1986 and plants were classified at maturity for sterility/fertility segregation. Sterile plants of *ms1 ms1* (T266) and the unknown mutant were observed for their pod-set under field conditions in 1985 and 1986.

Results. In the F_1 generation, 20 plants had normal pollen, 18 plants were characterized by large coenocytic pollen grains. This population gave a good fit to the expected 1:1 ratio, $\chi^2 = 0.106$, P = 0.75-0.50 (Table 1.) Twenty F_2 progenies segregated for sterility at maturity. In the F_2, 1,400 plants were fertile and 455 were sterile. The data gave a good fit to the expected 3:1 ratio and confirmed results of testcrosses in the F_1 generation (Table 1). Results indicated that independent mutation has occurred at the *ms1* locus.

Pod set observed on sterile plants of *ms1 ms1* (T266) and plants of the new mutation suggested that the new mutation was not as female fertile as *ms1* (T266). During two years of observations, plants of *ms1 ms1* (T266) averaged 2.0 seed per pod and give 13.2 seeds per plant. The mutant averaged 1.1 seed per pod and 4.4 seeds per plant.

We propose to name strain T268 as *ms1* (Ames 1) and strain carrying new mutation as *ms1* (Ames 2) (T287).

Table 1. Segregation for fertility/sterility in F_1 and F_2 population from cross *ms1 ms1* (T266) x *Ms1 ms1* unknown mutant

Generation	Number of plants			Expected ratio	χ^2	P
	Fertile	Sterile	Total			
F_1	20	18	38	1:1	0.106	0.70-0.50
F_2	1400	455	1855	3:1	0.220	0.70-0.50
Total (20 df)					5.075	>0.995
Homogeneity χ^2 (19 df)					4.855	>0.995

Table 2. Seed production on sterile plants of new mutant and *ms1 ms1* (T266)

	Year	Number of sterile plants	Average number	
			Seeds/plant	Seeds/pod
New mutant	1985	174	5.9	1.1
	1986	100	3.0	1.1
			4.84	1.1
ms1 ms1 (T266)	1985	32	14.9	2.1
	1986	50	11.6	2.0
			12.89	2.0

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8) Inheritance and derivation of T218H.

Genetic type T218M was found in the cultivar 'Illini' in 1952 at Urbana, Illinois. Genetic type T225M was found in the cultivar 'Lincoln' in Iowa before 1955. T218M phenotypically is similar to T225M in that an unstable allele results in a chlorophyll chimera. We derived T225H (*Y18 y18*) by crossing Lincoln as female parent by T225 a yellow plant (*y18 y18*) grown in the growth chamber, as male parent (Sheridan and Palmer, 1975). We also have transmitted the *y18* gamete in crosses by using flowers from a yellow branch on a variegated plant (Sheridan and Palmer, 1975). Our objective was to develop T218H.

Both T225H and T218H give predictable 3:1 phenotypic ratios and 1:2:1 genotypic ratios, upon self-pollination of heterozygotes. The recessive genotype is lethal under field conditions and is a conditional lethal in the greenhouse. Heterozygous plants are used in cross-pollinations in genetic studies.

We used Illini as the female parent and flowers from a mostly yellow branch on a variegated T218M plant as male parent. All nine F_1 plants were green as expected. In the F_2, two progenies were all green. Seven progenies segregated about 3 green:1 yellow plants (Table 1). Twenty F_2 plants within each of these seven families were threshed individually and were evaluated as F_2-plant-progeny rows. About two-thirds of the 140 entries segregated, as expected, for green and yellow plants (Table 1). The ratio of green:yellow plants in these segregating families was about 3:1 (Table 1).

The failure of all nine F_1 plants to segregate in the F_2 for green and yellow plants was not unexpected. In plants, in general, segregation patterns of chimeras manifest a relationship between sector phenotype and gamete genotype. When chimeric plants are used in crosses and the yellow trait is a single-gene recessive 1) flowers borne on nonchimeric green regions produce all green F_2 progeny, 2) flowers borne on sectored regions may produce all green progeny, green and variegated progeny, or about 3 green:1 yellow progeny, and 3) flowers borne on yellow regions produce all segregating progeny of about 3 green:1 yellow.

Hatfield and Palmer (1986) tested for allelism between T218H and T225H. F_1, F_2, and F_3 data reported by them confirmed the allelism of these two mutants. On the basis of these results, the heterozygote should be added to the Genetic Type Collection as T218H and the appropriate gene symbol should be *Y18 y18*.

Table 1. Inheritance and derivation of T218H. Parents were Illini x T218M

F_1 plant no.	No. F_2 plants		χ^2		F_2 families		χ^2		No. F_3 plants in seg. families		χ^2	
	Green	Yellow	(3:1)	P	Nonseg.	/Seg.	(1:2)	P	Green	Yellow	(3:1)	P
1	319	101	0.20	0.50-0.75	10	10	2.50	0.10-0.25	3201	1097	0.64	0.25-0.50
2	297	87	1.12	0.25-0.50	5	15	0.62	0.25-0.50	4455	1374	6.34	0.01-0.025
3	310*	0	0	0	20*	0	0	0	0	0	0	0
4	197*	0	0	0	20*	0	0	0	0	0	0	0
5	185	56	0.40	0.50-0.75	4	16	1.59	0.10-0.25	4931	1603	0.76	0.25-0.50
6	483	152	0.38	0.50-0.75	6	14	0.10	0.75	4713	1529	0.85	0.25-0.50
7	512	165	0.14	0.50-0.75	7	13	0.03	> 0.90	4127	1331	1.09	0.25-0.50
8	403	133	0.01	>0.90	6	14	0.10	0.75	4544	1503	0.07	0.75-0.90
9	369	119	0.10	0.75	7	13	0.03	>0.90	4380	1417	1.10	0.25-0.50
Total	2568	813	2.35	>0.90	45	95	4.97	0.75-0.50	30,351	9854	10.85	0.10-0.25
Pooled (1 df)			1.64	0.10-0.25			0.10	0.75			5.16	0.01-0.025
Homogeneity (6 df)			0.71	>0.90			4.87	0.50-0.75			5.69	0.25-0.50

*Not included in total.

Table 2. Description of T218M, T218H, T225M, and T225H

Strain	Gene	Phenotype	Source
T218M	*Y18-m*	Chlorophyll chimera (resembles T225M)	Found in Illini in 1952
T218H	*y18*	Near-lethal, yellow leaves	From T218M
T225M	*Y18-m*	Unstable allele resulting in chlorophyll chimera	Found in Lincoln in Iowa before 1955
T225H	*y18*	Near-lethal, yellow leaves	From T225M

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9) PI 153252 is a true-breeding yellow mutant.

We have searched for chromosome interchanges and inversions among progeny of accessions crossed with cultivars of standard chromosome structure. We make the cross-pollinations and examine the F_1 plants for fertility/sterility (Delannay et al., 1982; Palmer et al., 1987). If sterility is present, meiocytes are examined to confirm the presence of a chromosome aberration. We routinely plant about 50 F_2 seed from each F_1 plant to check segregation of marker genes to confirm hybridity.

In the cross of 'Swift' x PI 153252, the two F_1 plants were fertile. In the F_2, green plants and yellow plants were observed among progeny from both F_1 plants (Table 1). The yellow plant color became more evident in the new leaves when the plants were growing vigorously and the ambient temperature was increasing. We are not certain whether the yellow color of the emerging leaves was the result of plant age (developmental stage), ambient temperature, or their interaction. The yellow plants were quite vigorous.

Sixty-one F_2 plants from one F_1 family (A83-83-2) were chosen at random to advance to the F_3. Segregation for plant color in the F_3 fit a 1:2:1 ratio for all green:segregating : all yellow (Table 2). Allelism tests with PI 153252 yellow plants with known yellow mutants were not done.

Table 1. F_2 segregation for plant color of Swift x PI 153252

F_1 plant no.	No. F_2 plants		χ^2 3:1	P
	Green	Yellow		
A83-83-1	267	83	0.31	0.75-0.50
A83-83-2	325	114	0.22	0.75-0.50

Table 2. F_2 genotypes determined from F_3 plants of Swift x PI 153252

No. F_2 families			χ^2 3:1	P
All green	Segregating	All yellow		
15	31	15	0.02	0.99

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10) Chloroplast DNA analysis of *cyt-Y3*.

Introduction: The cytoplasmically inherited yellow foliar mutant, *cyt-Y3*, is the third uniparentally inherited mutant reported for soybeans (Shoemaker et al., 1985). The remaining two maternally inherited mutants are *cyt-Y2*, also a yellow foliar mutant (Palmer and Mascia, 1980), and *cyt-G1*, a green cotyledon mutant (Terao, 1918). *cyt-Y3* is unique in the respect that it is near-lethal under normal light conditions and lethal under elevated photosynthetic photon flux densities, i.e., 600 to 2,100 microeinsteins m^{-2}, respectively.

Uniparentally inherited mutations are difficult to evaluate by classical methods. Because soybean organelles are inherited predominantly, or solely, through the maternal parent (Sisson et al., 1978; Hatfield et al., 1985), the associated genes are inherited as a unit and no recombinational studies are possible. In fact, it is not yet possible to determine unambiguously whether the uniparentally inherited mutation (*cyt-Y3*) occurs within the chloroplast genome or the mitochondrial genome. Because the *cyt-Y3* mutation affects the chloroplast chlorophyll content and chloroplast ultrastructure, we postulate that the mutation lies within the ctDNA molecule.

The mutant, *cyt-Y3*, is assigned the Laboratory Genetic Type Collection Number T278. The mutant arose as a chimera. Subsequent selfings produced progeny that were yellow (*cyt-Y3*), green (*cyt-G3*), and chimera. Consequently, *cyt-G3* and *cyt-Y3* can be considered genetically identical with the exception of the mutation giving rise to the *cyt-Y3* phenotype. Therefore, any DNA sequence variation detected between plant populations expressing the two different phenotypes would indicate the presence of a molecular lesion responsible for the mutant phenotype and would provide a "tag" by which we might identify the location of the coding sequence of the altered gene.

The objective of the experiments described was to attempt to identify ctDNA sequence variation, using restriction endonucleases, between *cyt-G3* and *cyt-Y3* sib populations. Restriction endonucleases are enzymes that recognize and cleave DNA at specific sequences of four to seven base pairs in length. DNA can be digested with a particular restriction endonuclease, and the mixture of DNA fragments generated then separated by agarose gel electrophoresis. A characteristic pattern of bands can be visualized on the gel. Each band represents a length of DNA between restriction sites. On the basis of comparative electrophoretic mobilities, the number of base pairs constituting each fragment can be estimated. A restriction enzyme cleavage site can be considered a genetic marker and can be used to locate mutant lesions along a chromosome.

Materials and methods: Seed for the *cyt-Y3* and *cyt-G3* comparisons was increased in field plots in Ames, Iowa during summer, 1986. Due to the lethality of the *cyt-Y3* phenotype under normal environmental conditions, seed was increased only from the chimera plants. The yellow plants died as seedlings and green plants were rogued. Bulked seed from chimera plants was grown in the greenhouse sandbench during winter 1986-87 without supplemental lighting.

Trifoliates were harvested from green plants and from yellow plants. Enrichment of ctDNA was carried out according to published procedures (Shoemaker et al., 1984).

ctDNA was digested with the restriction endonucleases HpaII, ClaI, AvaI, XhoI and PstI. DNA fragments were electrophoresed in 0.8% agarose gels in a buffer of 90 mM Tris, 90 mM boric acid, and 2.5 mM Na_2 EDTA. Electrophoresis was conducted at room temperature for 18 hr at 50 v. Gels were stained in ethidium bromide solution (0.5 µg/ml) and photographed with a MP-4 camera apparatus over short-wave ultraviolet light using Polaroid Land Pack film Type 665 with Kodak Wratten gelatin filters No. 1A (U.V.) and No. 15 (orange).

Results and discussion: Deletions, insertions and other chromosomal rearrangements can alter the relative position of restriction sites, yielding shorter or longer fragments. A loss of a restriction site can occur due to a point mutation within the restriction site or from any chromosomal rearrangement resulting in an alteration of a recognition sequence. An increase in the number of restriction sites may also be observed as a consequence of insertions or rearrangements or base changes resulting in the creation of additional recognition sequences. These differences are reflected as alterations in the pattern of bands produced by gel electrophoresis, and are termed restriction fragment length polymorphisms, or RFLP's.

No RFLP's were observed between ctDNA prepared from *cyt-Y3* plants and *cyt-G3* plants using the restriction enzymes listed above. If, however, we make the assumption that the mutation responsible for the *cyt-Y3* phenotype does lie within the chloroplast genome, the failure to identify an RFLP still provides us with some valuable information. This suggests that the mutant phenotype arises either from a simple point mutation that is not detected with the restriction endonucleases used in this study, or from a relatively small addition, deletion, translocation or inversion that does not involve a restriction site. Studies of *cyt-Y3* are continuing in our laboratory by utilizing additional restriction endonucleases.

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